

THAT WHICH IS CLAIMED:

1. A plant that is genetically modified to reduce or eliminate the activity of one or more proteases in its protein storage tissue.

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2. The plant of claim 1 wherein said protein storage tissue is seed.

3. The plant of claim 1 wherein said protein storage tissue is selected from the group consisting of tubers, roots, and leaves.

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4. The plant according to claim 1 wherein said protease is selected from the group consisting of vacuolar processing enzymes, aspartic proteases, papain-type proteases, subtilisins, and serine endopeptidases.

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5. The plant according to claim 4 wherein said protease is selected from the group consisting of  $\alpha$ -vacuolar processing enzyme,  $\beta$ -vacuolar processing enzyme,  $\gamma$ -vacuolar processing enzyme,  $\epsilon$ -vacuolar processing enzyme, aspartic protease AP1, and aspartic protease AP2.

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6. The plant according to claim 1 wherein at least one protease has an amino acid sequence selected from the group consisting of:

a) the amino acid sequence encoded by the nucleic acid sequence of SEQ ID NO:1;

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b) the amino acid sequence of a fragment of the amino acid sequence set forth in SEQ ID NO:2 wherein said fragment comprises at least 10 contiguous amino acids of SEQ ID NO:2;

c) the amino acid sequence of a sequence variant of a polypeptide having the amino acid sequence set forth in SEQ ID NO:2 wherein said sequence variant has protease activity and is encoded by a nucleotide sequence that hybridizes to the

complement of the nucleotide sequence shown in SEQ ID NO:1 under stringent conditions; and,

5 d) the amino acid sequence of a sequence variant of a polypeptide having the amino acid sequence set forth in SEQ ID NO:2 wherein said sequence variant has protease activity and is encoded by a nucleotide sequence having at least 60 % sequence identity to the nucleotide sequence of SEQ ID NO:1.

e) the amino acid sequence set forth in SEQ ID NO:2.

10 7. The plant according to claim 1 wherein said plant is a dicot.

8. The plant according to claim 7 wherein said dicot is selected from the group consisting of *Arabidopsis*, soybean, sunflower, canola, cotton, and safflower.

15 9. The plant of claim 1 wherein said plant is a monocot.

10. The plant of claim 9 wherein said monocot is selected from the group consisting of maize, wheat, rice, barley, oats, rye, and millet.

20 11. Transformed seed obtained from the plant of claim 1.

12. The plant of claim 1 wherein said plant is transformed with an expression cassette comprising a promoter operably linked with a polypeptide of interest, said promoter selected from the group consisting of a constitutive promoter, a tissue-preferred promoter, a leaf-specific promoter, a root-preferred promoter, and a seed-preferred promoter.

25 13. A method for producing one or more polypeptides in a plant protein storage tissue comprising the steps of :

a) modifying the nucleotide sequence encoding said polypeptide to produce a modified nucleotide sequence encoding a polypeptide that will no longer be cleaved by one or more proteases, and

b) transforming an expression cassette comprising said modified nucleotide sequence into a plant wherein the expression cassette directs the expression of said polypeptide that will no longer be cleaved by one or more proteases in the protein storage tissue of said plant.

14. The method according to claim 13 wherein said plant protein storage tissue is selected from the group consisting of: seed, tubers, roots, and leaves.

15. The method of claim 14 wherein said polypeptides are selected from the group consisting of insulin, human growth hormone, pepsin, cellulases, pectinases, hemicellulases, phytases, hydrolases, esterases, peroxidases, fibrinogen, plasma proteins, serum albumin, factor IX, factor XIII, thrombin, protein C, xylanase, isoamylase, glucoamylase,  $\alpha$ -amylase, lysozyme, catalase,  $\beta$ -glucanase,  $\beta$ -casein, lactase, urease, glucose isomerase, superoxide dismutase, pullulanase, invertase, streptavidin, avidin, alkaline phosphatase, aprotinin,  $\beta$ -glucuronidase, protease inhibitors, aprotinin, pepsin, chymotrypsin, trypsin, papain, kinases, phosphatases, antibodies, deoxyribonucleases, ribonucleases, phospholipases, lipases, peptide hormones, green fluorescent protein, secreted antibodies, single chain antibodies, spider silk, soybean 2S albumin, glycinin, and  $\beta$ -conglycinin.

16. The method of claim 13 wherein said proteases are selected from the group consisting of vacuolar processing enzymes, aspartic proteases, papain-type proteases, subtilisins, and serine endopeptidases.

17. The method of claim 16 wherein said protease is selected from the group consisting of  $\alpha$ -vacuolar processing enzyme,  $\beta$ -vacuolar processing enzyme,  $\gamma$ -vacuolar

processing enzyme,  $\epsilon$ -vacuolar processing enzyme, aspartic protease AP1, and aspartic protease AP2.

18. A plant produced by the method of claim 13.

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19. A method for increasing the accumulation of one or more polypeptides of interest in seed, said method comprising genetically modifying a plant to alter the activity of at least one protease in the seed of said plant.

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20. The method of claim 19 wherein the magnitude of the alteration of the activity of the protease is at least 2 fold.

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21. The method of claim 19 wherein said seed protease is selected from the group consisting of vacuolar processing enzymes, papain-type proteases, subtilisins, and serine endopeptidases.

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22. The method according to claim 21 wherein said protease is selected from the group consisting of:  $\alpha$ -vacuolar processing enzyme,  $\beta$ -vacuolar processing enzyme,  $\gamma$ -vacuolar processing enzyme,  $\epsilon$ -vacuolar processing enzyme, aspartic protease AP1, and aspartic protease AP2.

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23. The method according to claim 19 wherein the activity of said protease is decreased.

24. The method according to claim 19 wherein the activity of said protease is increased.

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25. The method of claim 19 wherein the step of genetically modifying a plant to alter the activity of at least one protease in the seed of said plant comprises transforming said plant with an expression cassette comprising a promoter operably

linked with a polypeptide of interest, wherein said promoter is selected from the group consisting of a constitutive promoter, a tissue-preferred promoter, a leaf-specific promoter, a root-preferred promoter, and a seed-preferred promoter.

5           26.    A polypeptide produced by the method of claim 13.

          27.    The polypeptide of claim 26, wherein said polypeptide is a soy protein isolate.

10           28.    A method for increasing the accumulation of one or more polypeptides in plant protein storage tissue, comprising the steps of :

                  a)    modifying the nucleotide sequence encoding said polypeptide to produce a modified nucleotide sequence encoding a polypeptide that will no longer be cleaved by one or more proteases, and

15                   b)    transforming an expression cassette comprising said modified nucleotide sequence into a plant, wherein the expression cassette directs the expression of said polypeptide in protein storage tissue produced by said plant.

          29.    The method according to claim 28 wherein said protein storage tissue is  
20    selected from the group consisting of: seed, tubers, roots, and leaves.

          30.    The method according to claim 28 wherein said seed protease is selected from the group consisting vacuolar processing enzymes, papain-type proteases, aspartic proteases, subtilisins, and serine endopeptidases.

25           31.    The method according to claim 30 wherein at least one of said proteases is selected from the group consisting of:  $\alpha$ -vacuolar processing enzyme,  $\beta$ -vacuolar processing enzyme,  $\gamma$ -vacuolar processing enzyme,  $\epsilon$ -vacuolar processing enzyme, aspartic protease AP1, and aspartic protease AP2.

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32. A method for preventing the proteolytic degradation of one or more polypeptides in plant protein storage tissues, comprising the steps of :

- 5 a) modifying the nucleotide sequence encoding said polypeptide to produce a modified nucleotide sequence encoding a polypeptide that will no longer be cleaved by one or more proteases, and
- b) transforming an expression cassette comprising said modified nucleotide sequence into a plant wherein the expression cassette directs the expression of said polypeptide in protein storage tissue produced by said plant.

10 33. The method of claim 32 wherein said plant protein storage tissue is selected from the group consisting of seed, tubers, roots, and leaves.

15 34. The method of claim 32 wherein said seed proteases are selected from the group consisting of vacuolar processing enzymes, aspartic proteases, papain-type proteases, subtilisins, and serine endopeptidases.

20 35. The method according to claim 34 wherein said proteases is selected from the group consisting of  $\alpha$ -vacuolar processing enzyme,  $\beta$ -vacuolar processing enzyme,  $\gamma$ -vacuolar processing enzyme,  $\epsilon$ -vacuolar processing enzyme, aspartic protease AP1, aspartic protease AP2.

36. An isolated nucleic acid molecule comprising a nucleotide sequence selected from the group consisting of:

- 25 a) the nucleotide sequence set forth in SEQ ID NO:1,
- b) a nucleic acid molecule encoding the amino acid sequence set forth in SEQ ID NO:2;
- c) a nucleotide sequence having at least 60% sequence identity to the nucleotide sequence of SEQ ID NO:1 wherein said nucleotide sequence encodes a polypeptide having protease activity and,

d) a nucleotide sequence encoding a fragment of the amino acid sequence shown in SEQ ID NO:2 wherein the fragment comprises at least 10 contiguous amino acids of SEQ ID NO:2;

5 e) a nucleotide sequence that hybridizes under stringent conditions to the complement of the nucleotide sequence set forth in SEQ ID NO:1 wherein said stringent conditions comprise hybridization in 50% formamide, 1M NaCl, 1% sodium dodecyl sulphate at 37° C, and a wash in 15 mM NaCl, 1.5 mM trisodium citrate at 60°C.

f) the complement of a nucleotide sequence in a), b), c), d), or e).

10 37. An isolated polypeptide comprising an amino acid sequence selected from the group consisting of:

a) the amino acid sequence encoded by the nucleic acid sequence of SEQ ID NO:1;

15 b) the amino acid sequence of a fragment of the amino acid sequence set forth in SEQ ID NO:2 wherein said fragment comprises at least 10 contiguous amino acids of SEQ ID NO:2;

c) the amino acid sequence of a sequence variant of a polypeptide having the amino acid sequence set forth in SEQ ID NO:2 wherein said sequence variant has protease activity and is encoded by a nucleotide sequence that hybridizes to the  
20 complement of the nucleotide sequence shown in SEQ ID NO:1 under stringent conditions; and,

d) the amino acid sequence of a sequence variant of a polypeptide having the amino acid sequence set forth in SEQ ID NO:2 wherein said sequence variant has protease activity and is encoded by a nucleotide sequence having at least 60 %  
25 sequence identity to the nucleotide sequence of SEQ ID NO:1.

e) the amino acid sequence set forth in SEQ ID NO:2.